REMARKS

Applicant wishes to thank the Examiner Shubo Zhou for the courtesy extended to the Applicant's representatives Daniel Hart and Marina Gordey by granting a telephonic interview on November 29, 2006. Claims 1, 9, 10, 14, 23 and 24 have been amended. Support for the amendment can be found in the Specification in paragraphs 36 and 43. Claims 1, 3-33 are presented for the examination. The following addresses the substance of the Office Action.

Priority

The Examiner indicated that the filed copies of the priority documents: EP99870106.4 and EP00870025.4 are not certified copies. Since the mailing of this Office Action, Applicant received Priority Acknowledgment, mailed July 17, 2006, indicating that priority documents submitted under 35 U.S.C. 119 were received and placed of record in the file.

Non-obviousness

The Examiner has rejected Claims 1, 3-6, 9, 10, 28, and 33 under 35 U.S.C. §103(a) as being allegedly unpatentable over Lockhart et al. (USP 6,344,316) in view of Hacker et al. ("Immunogold-silver staining – autometallography: recent developments and protocols" in: "Analytical Methodology: Theory, Applications & Protocols", Eds. Jiang Gu, Eaton Publishing, Chapter 2, pages 41-54, 1997). Specifically, the Examiner asserted that it would have been obvious at the time the invention was made to one of ordinary skill in the art to modify the method of Lockhart et al. to adopt the autometallography (AMG) method of Hacker et al. for detection of nucleic acids in a sample using microarray to take AMG's advantage of being highly specific and extremely sensitive.

> To establish a *prima facie* case of obviousness a three-prong test must be met. First, there must be some suggestion or motivation, either in the references or in the knowledge generally available among those of ordinary skill in the art, to modify the reference. Second, there must be a reasonable expectation of success found in the prior art. Third, the prior art must reference must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991).

Here, one skilled in the art would not be motivated to produce the claimed invention. Furthermore, the claimed invention provides unexpected advantages.

The currently amended claim 1 recites that the possible presence of precipitate(s) in discrete region(s) is determined and quantified. The gist of the present invention resides in

detection and/or quantification of a target compound by determining the possible presence and quantification of precipitate in discrete regions of an array.

As attested in the Declaration by the inventor, Jose Remacle, filed herewith under 37 C.F.R §1.132, although both silver staining technology and microarray technology coexisted for many years, those skilled in the art had never been motivated to combine these technologies prior to the presently claimed invention. Gold and/or silver staining had been used in the context of blotting for as long as 20 years. Microarray technology was developed more than 15 years ago. However, rather than using catalytically generated metallic precipitates as in the presently claimed invention, those working in the microarray field had used fluorescence to detect target molecules bound to the microarray. Thus, despite their coexistence for many years, prior to the present invention, those skilled in the microarray field were not motivated to utilize metallic precipitates generated by catalytic reduction for detecting target molecules bound to microarrays.

It is only after this invention was made, that others recognized the advantages of this technology over the fluorescence methods which dominated the field at the time. As discussed in paragraphs 6 and 7 of the 1.132 Declaration of Dr. Remacle, the post-filing date publications showed that the novel method invented by the present inventors is 100 times more sensitive than the fluorescence-based method. Many of the references published after the priority date of the present application listed in this 1.132 Declaration not only recognized the advantages of using metallic precipitates generated by catalytic reduction to detect target molecules bound to microarrays and further demonstrated the importance of such technology to the microarray field, but also made specific reference to a publication by Dr. I. Alexandre, the main inventor of this application, entitled "Colorimetric Silver Detection of DNA Microarrays", *Anal. Biochem.* 295:1-8 (2001).

In addition, the Declaration of Dr. Bing, who is not an applicant on the present application and would not financially benefit from its issuance, sets forth further advantages of the present invention. In particular, as attested in his Declaration, in Dr. Bing's personal experience, relative to fluorescence based arrays the arrays of the present invention showed such unexpected advantages as being user-friendly, requiring fewer method steps, allowing the use of less expensive equipment (i.e. no need for an electron microscope). Dr. Bing also noted that,

unlike fluorescence based microarray techniques, the present microarrays can be analyzed by a lab technician rather than requiring an expert to operate the detection apparatus. .

Thus, the use of metallic precipitates generated by catalytic reduction to detect target molecules bound to microarrays provides significant unexpected advantages over fluorescence based methods.

Furthermore, Applicants maintain that one skilled in the art would not be motivated to combine Hacker and Lockhart. Lockhart et al. disclose the detection of binding between targets and capture molecules on high density arrays. Lockhart et al. further disclose the use of a colorimetric label (e.g., colloidal gold) of about 40-80 nm in size and capable of scattering green light. Gold particles of 40-80 nm are highly suitable for detection by resonance light scattering (RLS), but are not compatible with catalytic reduction of metal such as silver which requires smaller particle size of equal or lower that 20 nm. The reason is that gold particles can only react if they are of around the same size as the biological molecules, i.e. a few nanometers. If too large, the reaction of catalytic reduction becomes slow or does not take place. In fact, the Examiner agreed during the interview on April 21, 2004, that Lockhart et al. do not teach or suggest the catalytic reduction of a metal present in solution.

Additionally, the use of gold particles is disclosed in a long list of different possibilities, which demonstrates that this labeling technique is not the preferred embodiment. Indeed, the proposed examples in Lockhart et al. make use of fluorescence labels which were the dominating labels for microarrays in those days (see page 24, lines 37-53).

Hacker et al. disclose the use of antibodies having gold particles fixed thereto along with silver intensification in the context of localizing a molecule in a cell or tissue, the context being significantly different from the microarrays utilized in the presently claimed invention. Moreover, Hacker et al. teaches the use of small gold particles of about 5 nm for optimal silver enhancement (page 45, second paragraph), confirming that gold particles of 40-80 nm, described by Lockhart are out of the suitable range for silver enhancement.

Hacker et al. mainly used the silver staining for visualization of specific nucleic acids and proteins in light and electron microscopic studies (see figures 1-4). In addition, Hacker et al. teach that some immunogold reagents available on the market do not produce high labeling density and generate an unacceptable background labeling. When high-quality products are used,

one still needs to check and optimize every step of the procedure (see page 49, first paragraph). Therefore, the method of Hacker et al. would not motivate a skilled artisan to use it for quantitative purposes, and this method has only been used for qualitative detection and not for quantitative purpose, as in the presently claimed invention.

Furthermore, in his Declaration Dr. Bing attests that as of 1999 the use of silver enhancement for *in situ* localization of target molecules was thought to result in variable sizes of precipitates, to produce background signals, to be non-quantitative, and to require electron microscopy for detection of the silver precipitate. Therefore, such limitations would suggest that silver enhancement would not be suitable for detecting target molecules bound to microarrays which require consistent precipitate sizes to enable accurate quantitation of the precipitate and thus of target molecules and comparison of targets bound to different portions of an array or to different arrays. In addition, electron microscopy is not a suitable detection methodology for microarray analysis because it requires skilled personnel, is time consuming and is not automatable.

Applicants maintain that, in view of the understanding of those skilled in the art as of 1999 that *in situ* silver enhanced had the foregoing characteristics which suggest that such methodology would not be compatible with high throughput analysis of target molecules bound to microarrays, those skilled in the art would not be motivated to combine Hacker and Lockhart.

Furthermore, Applicants maintain that the totality of the evidence presented to date regarding the use of silver enhancement for *in situ* analysis of cells suggests one skilled in the art as of the priority date of the present application would not be motivated to utilize such methodology in the context of high throughput microarray analysis. According to MPEP 2145:

> The totality of the prior art must be considered, and proceeding contrary to accepted wisdom in the art is evidence of nonobviousness. *In re Hedges*, 783 F.2d 1038, 228 USPQ 685 (Fed. Cir. 1986).
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> Furthermore, "[k]nown disadvantages in old devices which would naturally discourage search for new inventions may be taken into account in determining obviousness." *United States v. Adams*, 383 U.S. 39, 52, 148 USPQ 479, 484 (1966).

Applicants have previously successfully argued over another combination of Lockhart et al. with a reference which also taught silver enhancement of immunogold staining (Roth et al. USP 6,344,613) (see Amendment filed June 15, 2004). There, Roth stated that silver

enhancement of immunogold staining was not suitable for quantitative purposes, thus also teaching away from the presently claimed invention. As the Applicant argued, Roth et al. teach that methods using gold particels and silver intensification are undesirable or labor intensive in quantitative analyses. Roth's teaching away from the present invention was acknowledged by the Examiner and Applicants were sent a Notice of Allowance. However, subsequently Applicants received the present Office Action containing the rejection based on the combination of Hacker and Lockhart.

Just as Roth taught away from the combination of *in situ* silver enhancement with Lockhart, Dr. Bing in his 1.132 Declaration also states that at the time this invention was made that the use of silver enhancement for *in situ* localization of target molecules as of 1999 was thought to result in variable sizes of precipitates, to produce background signals, to be non-quantitative, and to require electron microscopy for detection of the silver precipitate, therefore suggesting that silver enhancement would not be amenable to microarray analysis.

Applicants thus maintain that the totality of the evidence suggest that, as of the priority date of the present application, those skilled in the art believed that several aspects of in situ silver enhancement methodology rendered such methodology incompatible with use in the context of high throughput microarray analysis. Accordingly, Applicants maintain that one skilled in the art would not have been motivated to combine Hacker and Lockhart.

For all of the above reasons, Claims 1, 3-6, 9, 10, 28, and 33 are non-obvious over the combination of cited references, and their rejection under 35 USC §103(a) should be withdrawn.

The Examiner has rejected Claims 7, 8, 11-27, 29, 31 and 32 under 35 U.S.C. §103(a) as being allegedly unpatentable over Lockhart et al. (USP 6,344,316) in view of Hacker et al., as applied to Claims 1, 3-6, 9, 10, 28, and 33 above, in view of Abouzied et al (*J. AOAC Internat.* 77:495-501). Specifically, the Examiner asserted that it would have been obvious at the time the invention was made to one of ordinary skill in the art to modify the methods of Lockhart et al. and Hacker et al. to use the microarray technology for detection of, not only nucleic acids, but also proteins/antibodies and/or receptors/ligands – to take advantage of microarray's universal utilities in detection multianalytes as disclosed by Abouzied et al.

As asserted above, the method of Claim 1 is non-obvious over the combination of Lockhart and Hacker. Claims 7, 8, 11-27, 29, 31 and 32 depend on Claim 1.

As discussed in the response to Office Action, filed on June 15, 2004, Abouzied et al. discloses a colorimetric method for screening and detecting analytes on nitrocellulose (NC) strips. In the method of Abouzied, a colored reaction product formed through the action of an enzyme linked to the target analyte is used to detect the presence of the analyte in the sample. Abouzied does not teach or suggest methods in which a metallic precipitate is formed by catalytic reduction of a metal. The method of detection includes visually comparing color intensities formed by precipitates on the NC membrane and quantitatively assaying line density using a CCD camera. In addition, in the method of Abouzied, the lines on the NC membrane strips are spaced 0.25 cm apart (page 496, col. 2). As indicated in the present Specification at page 2, line 32 through page 3, line 11, colorimetric assays in which an enzyme generates a colored reaction product which forms a precipitate, such as in the methods described in Abouzied, are unsuitable for use in arrays comprising a density of at least 20 discrete regions per cm^2 because the precipitate occupies an area which is too large to allow it to be localized to one or more discrete regions. This was acknowledged in the Office Action, mailed February 22, 2001 (see page 5), where the Examiner acknowledged that "...the dimension of the line blots disclosed does not allow a density of 20 or more discrete regions per cm^2 with each region having one species of capture molecule..." Therefore, Abouzied fails to cure the deficiencies of the primary references.

For these reasons, Claims 7, 8, 11-27, 29, 31 and 32 are non-obvious over the combination of the 3 cited references, and their rejection under 35 USC §103(a) should be withdrawn.

The Examiner has rejected Claims 29 and 30 under 35 U.S.C. §103(a) as being allegedly unpatentable over Lockhart et al. (USP 6,344,316) and Hacker et al. in view of Abouzied et al., as applied to Claims 1, 3-6, 9, 10, 28, and 33 above, and further in view of Gingeras et al. (USP 6,228,575). Specifically, the Examiner asserted that it would have been obvious at the time the invention was made to one of ordinary skill in the art to modify the method as disclosed in combination by Lockhart et al., Hacker et al. and Abouzied et al. to use the simple method of bar code and bar code reader of Gingeras et al. to take advantage of its convenience and speed.

As discussed above, Claim 14 (from which claims 29 and 30 depend) recites metallic precipitates formed by catalytic reduction of a metal. None of the cited primary references teach

or suggest that the recited metallic precipitates formed by catalytic reduction of a metal in one or more discrete regions are used for quantitation of the resulting signal. Gingeras fails to cure these deficiencies of the primary references.

Therefore, Claims 29 and 30 are non-obvious of the combination of the cited references, and their rejection under 35 USC §103(a) should be withdrawn.

CONCLUSION

Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, amendments to the claims, the reasons therefor, and arguments in support of the patentability of the pending claim set are presented above. In light of the above amendments and remarks, reconsideration and withdrawal of the outstanding rejections is specifically requested. If the Examiner finds any remaining impediment to the prompt allowance of these claims that could be clarified with a telephone conference, the Examiner is respectfully requested to initiate the same with the undersigned.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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